

### ***Listing of the Claims***

This listing of claims will replace all prior versions, and listings of claims in the application.

1 - 175. (Canceled)

176. (Currently amended) A composition comprising mouse embryonic stem cells and a serum-free cell culture medium ~~capable of preventing~~ which prevents differentiation of the mouse embryonic stem cells during expansion of the embryonic stem cells, wherein the serum-free cell culture medium comprises a lipid-rich serum albumin and comprises at least one factor selected from the group consisting of a leukemia inhibiting factor, a steel factor, a ciliary neurotrophic factor and an oncostatin M.

177. (Previously presented) The composition of claim 176,  
wherein the composition is capable of being stored indefinitely at less than or equal to about  $-135^{\circ}\text{C}$ , and

wherein embryonic stem cells of the composition can be cultivated after storing the composition at less than or equal to about  $-135^{\circ}\text{C}$ .

178 - 179. (Canceled)

180. (Previously presented) The composition of claim 176, wherein the albumin is bovine albumin.

181 - 182. (Canceled)

183. (Previously presented) The composition of claim 176, wherein the serum-free cell culture medium does not contain leukemia inhibitory factor.

184. (Previously presented) The composition of claim 176, wherein the at least one factor is leukemia inhibiting factor.

185. (Previously presented) The composition of claim 176, wherein the at least one factor is steel factor.

186. (Previously presented) The composition of claim 176, wherein the at least one factor is neurotrophic factor.

187. (Previously presented) The composition of claim 176, wherein the at least one factor is oncostatin M.

188. (Previously presented) The composition of claim 176,  
wherein the serum-free cell culture medium comprises a basal cell culture medium,  
wherein the basal cell culture medium comprises a serum-free supplement, and  
wherein the serum-free supplement comprises an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, or a trace element.

189. (Previously presented) The composition of claim 188,  
wherein the composition is capable of being stored indefinitely at less than or equal to about  $-135^{\circ}\text{C}$ , and  
wherein embryonic stem cells of the composition can be cultivated after storing the composition at less than or equal to about  $-135^{\circ}\text{C}$ .

190 - 191. (Canceled)

192. (Previously presented) The composition of claim 188, wherein the serum-free cell culture medium comprises an amino acid, a vitamin, a transferrin, an antioxidant, an insulin, a collagen precursor, and a trace element.

193 - 194. (Canceled)

195. (Previously presented) The composition of claim 188, wherein the serum-free cell culture medium does not contain leukemia inhibitory factor.

196. (Previously presented) The composition of claim 188, wherein the at least one factor is leukemia inhibiting factor.

197. (Previously presented) The composition of claim 188, wherein the at least one factor is steel factor.

198. (Previously presented) The composition of claim 188, wherein the at least one factor is ciliary neurotrophic factor.

199. (Previously presented) The composition of claim 188, wherein the at least one factor is oncostatin M.

200. (Previously presented) The composition of claim 188, wherein the albumin is bovine albumin.

201. (Previously presented) The composition of claim 188, wherein the albumin is human albumin.

202 - 203. (Canceled)

204. (Previously presented) The composition of claim 188, wherein the transferrin is bovine transferrin.

205. (Previously presented) The composition of claim 188, wherein the transferrin is human transferrin.

206. (Previously presented) The composition of claim 188, wherein the transferrin is iron-saturated.

207. (Previously presented) The composition of claim 188, wherein the insulin is bovine insulin.

208. (Previously presented) The composition of claim 188, wherein the insulin is human insulin.

209. (Previously presented) The composition of claim 188, wherein the insulin is recombinant insulin.

210. (Currently amended) A composition comprising mouse embryonic stem cells and a serum-free cell culture medium,

wherein the serum-free cell culture medium is obtained by combining a basal cell culture medium with a serum-free supplement,

wherein the serum-free supplement comprises a lipid-rich serum albumin and at least one component selected from the group consisting of an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, and a trace element,

wherein the composition comprises at least one factor selected from the group consisting of a leukemia inhibiting factor, a steel factor, a ciliary neurotrophic factor and an oncostatin M, and

wherein the serum-free cell culture medium ~~is capable of preventing~~ prevents differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

211. (Previously presented) The composition of claim 210,  
wherein the composition is capable of being stored indefinitely at less than or equal to about  $-135^{\circ}\text{C}$ , and

wherein embryonic stem cells of the composition can be cultivated after storing the composition at less than or equal to about  $-135^{\circ}\text{C}$ .

212 - 213. (Canceled)

214. (Previously presented) The composition of claim 210, wherein the serum-free cell culture medium comprises an amino acid, a vitamin, a transferrin, an antioxidant, an insulin, a collagen precursor, and a trace element.

215 - 216. (Canceled)

217. (Previously presented) The composition of claim 210, wherein the serum-free cell culture medium does not contain leukemia inhibitory factor.

218. (Previously presented) The composition of claim 210, wherein the at least one factor is leukemia inhibiting factor.

219. (Previously presented) The composition of claim 210, wherein the at least one factor is steel factor.

220. (Previously presented) The composition of claim 210, wherein the at least one factor is ciliary neurotrophic factor.

221. (Previously presented) The composition of claim 210, wherein the at least one factor is oncostatin M.

222. (Previously presented) The composition of claim 210, wherein the albumin is bovine albumin.

223. (Previously presented) The composition of claim 210, wherein the albumin is human albumin.

224-225. (Canceled)

226. (Previously presented) The composition of claim 210, wherein the transferrin is bovine transferrin.

227. (Previously presented) The composition of claim 210, wherein the transferrin is human transferrin.

228. (Previously presented) The composition of claim 210, wherein the transferrin is iron-saturated.

229. (Previously presented) The composition of claim 210, wherein the insulin is bovine insulin.

230. (Previously presented) The composition of claim 210, wherein the insulin is human insulin.

231. (Previously presented) The composition of claim 210, wherein the insulin is recombinant insulin.

232. (Currently amended) A product of manufacture comprising a container means, wherein the container means contains mouse embryonic stem cells and a serum-free supplement,

wherein the serum-free supplement comprises a lipid-rich serum albumin and at least one component selected from the group consisting of an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, and a trace element, and

wherein a basal cell culture medium supplemented with the serum-free supplement is ~~capable of preventing~~ prevents differentiation of the embryonic stem cells during expansion of the embryonic stem cells when the basal cell culture medium supplemented with the serum-free supplement comprises at least one factor selected from the group consisting of a leukemia inhibiting factor, a steel factor, a ciliary neurotrophic factor and an oncostatin M.

233. (Previously presented) The product of manufacture of claim 232 in a frozen state.

234. (Previously presented) The product of manufacture of claim 232 not containing leukemia inhibitory factor.

235. (Previously presented) The product of manufacture of claim 232 containing leukemia inhibiting factor.

236. (Previously presented) The product of manufacture of claim 232 containing steel factor.

237. (Previously presented) The product of manufacture of claim 232 containing ciliary neurotrophic factor.

238. (Previously presented) The product of manufacture of claim 232 containing oncostatin M.

239. (Previously presented) The product of manufacture of claim 232, wherein the albumin is bovine albumin.

240. (Previously presented) The product of manufacture of claim 232, wherein the albumin is human albumin.

241 - 242. (Canceled)

243. (Previously presented) The product of manufacture of claim 232, wherein the transferrin is bovine transferrin.

244. (Previously presented) The product of manufacture of claim 232, wherein the transferrin is human transferrin.

245. (Previously presented) The product of manufacture of claim 232, wherein the transferrin is iron-saturated.

246. (Previously presented) The product of manufacture of claim 232, wherein the insulin is bovine insulin.

247. (Previously presented) The product of manufacture of claim 232, wherein the insulin is human insulin.

248. (Previously presented) The product of manufacture of claim 232, wherein the insulin is recombinant insulin.

249. (Currently amended) A product of manufacture comprising a container means, wherein the container means contains mouse embryonic stem cells and a serum-free cell culture medium,

wherein the serum-free cell culture medium comprises a basal cell culture medium, wherein the basal cell culture medium is supplemented with a serum-free supplement, wherein the serum-free supplement comprises a lipid-rich serum albumin and at least one component selected from the group consisting of an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, and a trace element, and

wherein the serum-free cell culture medium ~~is capable of preventing~~ prevents differentiation of the embryonic stem cells during expansion of the embryonic stem cells when the serum-free cell culture medium comprises at least one factor selected from the group consisting of a leukemia inhibiting factor, a steel factor, a ciliary neurotrophic factor and an oncostatin M.

250. (Canceled)



251. (Currently amended) A product of manufacture comprising a first container means and a second container means,

wherein the first container means contains a serum-free supplement,

wherein the second container means contains mouse embryonic stem cells,

wherein the serum-free supplement comprises a lipid-rich serum albumin and at least one component selected from the group consisting of an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, and a trace element, and

wherein a basal cell culture medium supplemented with the serum-free supplement and at least one factor selected from the group consisting of a leukemia inhibiting factor, a steel factor, a ciliary neurotrophic factor and an oncostatin M ~~is capable of preventing~~ prevents differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

252. (Previously presented) The product of manufacture of claim 251 further comprising a third container means containing a basal medium.

253. (Currently amended) A product of manufacture comprising a first container means and a second container means,

wherein the first container means contains a serum-free cell culture medium,

wherein the second container means contains mouse embryonic stem cells,

wherein the serum-free cell culture medium comprises a basal cell culture medium,

wherein the basal cell culture medium is supplemented with a serum-free supplement,

wherein the serum-free supplement comprises a lipid-rich serum albumin and at least one component selected from the group consisting of an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, and a trace element, and

wherein the serum-free cell culture medium ~~is capable of preventing~~ prevents differentiation of the embryonic stem cells during expansion of the embryonic stem cells when the serum-free cell culture medium comprises at least one factor selected from the group

consisting of a leukemia inhibiting factor, a steel factor, a ciliary neurotrophic factor and an oncostatin M.

254. (Canceled)

255. (Currently amended) A method for expanding mouse embryonic stem cells comprising contacting the embryonic stem cells with a serum-free cell culture medium ~~capable of preventing which prevents~~ differentiation of the embryonic stem cells during expansion of the embryonic stem cells, wherein the serum-free cell culture medium comprises a lipid-rich serum albumin and at least one factor selected from the group consisting of a leukemia inhibiting factor, a steel factor, a ciliary neurotrophic factor and an oncostatin M.

256. (Previously presented) The method of claim 255 further comprising seeding the embryonic stem cells upon a layer of feeder cells.

257. (Previously presented) The method of claim 256, wherein the feeder cells are primary embryonic fibroblasts, inactivated feeder cells, or STO cells.

258. (Previously presented) The method of claim 255, wherein the serum-free cell culture medium does not contain leukemia inhibitory factor.

259. (Previously presented) The method of claim 255, wherein the at least one factor is leukemia inhibiting factor.

260. (Previously presented) The method of claim 255, wherein the at least one factor is steel factor.

261. (Previously presented) The method of claim 255, wherein the at least one factor is ciliary neurotrophic factor.

262. (Previously presented) The method of claim 255, wherein the at least one factor is oncostatin M.

263. (Currently amended) A method for expanding mouse embryonic stem cells in a serum-free cell culture medium comprising

- (a) contacting the embryonic stem cells with a serum-free cell culture medium, and
- (b) expanding the embryonic stem cells,

wherein the serum-free cell culture medium comprises a basal cell culture medium; at least one factor selected from the group consisting of a leukemia inhibiting factor, a steel factor, a ciliary neurotrophic factor and an oncostatin M; and a serum-free supplement,

wherein the serum-free supplement comprises a lipid-rich serum albumin and at least one component selected from the group consisting of an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, and a trace element, and

wherein the serum-free cell culture medium ~~is capable of preventing~~ prevents differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

264. (Previously presented) The method of claim 263, wherein the albumin is bovine albumin.

265. (Previously presented) The method of claim 263, wherein the albumin is human albumin.

266-267. (Canceled)

268. (Previously presented) The method of claim 263, wherein the transferrin is bovine transferrin.

269. (Previously presented) The method of claim 263, wherein the transferrin is human transferrin.

270. (Previously presented) The method of claim 263, wherein the transferrin is iron-saturated.

271. (Previously presented) The method of claim 263, wherein the insulin is bovine insulin.

272. (Previously presented) The method of claim 263, wherein the insulin is human insulin.

273. (Previously presented) The method of claim 263, wherein the insulin is recombinant insulin.

274. (Currently amended) A method for preventing the differentiation of mouse embryonic stem cells in a serum-free cell culture medium comprising

- (a) contacting the embryonic stem cells with a serum-free cell culture medium, and
- (b) expanding the embryonic stem cells,

wherein the serum-free cell culture medium comprises a basal cell culture medium; at least one factor selected from the group consisting of a leukemia inhibiting factor, a steel factor, a ciliary neurotrophic factor and an oncostatin M; and a serum-free supplement,

wherein the serum-free supplement comprises a lipid-rich serum albumin and at least one component selected from the group consisting of an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, and a trace element, and

wherein the serum-free cell culture medium ~~is capable of preventing~~ prevents differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

275-277. (Canceled)

278. (Currently amended) A method for obtaining mouse embryonic stem cells in a serum-free cell culture medium comprising

- (a) isolating the embryonic stem cells from blastocysts, and

(b) expanding the embryonic stem cells in a serum-free cell culture medium,  
wherein the serum-free cell culture medium comprises a basal cell culture medium,  
wherein the serum-free cell culture medium comprises at least one factor selected from  
the group consisting of a leukemia inhibiting factor, a steel factor, a ciliary neurotrophic factor  
and an oncostatin M,

wherein the basal cell culture medium is supplemented with a serum-free supplement,  
wherein the serum-free supplement comprises a lipid-rich serum albumin and at least one  
component selected from the group consisting of an amino acid, a vitamin, a transferrin, a  
transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, and a  
trace element, and

wherein the serum-free cell culture medium ~~is capable of preventing~~ prevents  
differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

279. (Canceled)

280. (Currently amended) A method for producing a recombinant protein in mouse  
embryonic stem cells in a serum-free cell culture medium comprising

(a) obtaining a recombinant mouse embryonic stem cell containing a nucleic acid  
molecule encoding a recombinant protein,

(b) contacting the recombinant embryonic stem cell with a serum-free cell culture  
medium,

(c) expanding the recombinant embryonic stem cell in the serum-free cell culture medium  
to form a population of recombinant embryonic stem cells, and

(d) isolating the recombinant protein from the population of recombinant embryonic stem  
cells or from the serum-free cell culture medium of (c),

wherein the basal cell culture medium is supplemented with a serum-free supplement,  
wherein the serum-free supplement comprises a lipid-rich serum albumin and at least one  
component selected from the group consisting of an amino acid, a vitamin, a transferrin, a  
transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, and a  
trace element,

wherein the serum-free cell culture medium comprises at least one factor selected from the group consisting of a leukemia inhibiting factor, a steel factor, a ciliary neurotrophic factor and an oncostatin M, and

wherein the serum-free cell culture medium ~~is capable of preventing~~ prevents differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

281. (Previously presented) The method of claim 280 wherein the recombinant protein is isolated from the population of recombinant embryonic stem cells.

282. (Previously presented) The method of claim 280, wherein the recombinant protein is isolated from the serum-free cell culture medium of (c).